27. (NEW) A nucleic acid according to claim 26, wherein the carbohydrate modifying enzyme is selected from the group consisting of a galactosyl sulphating enzyme and a phosphorylating enzyme.

28. (NEW) A nucleic acid encoding a chimeric enzyme, wherein said chimeric enzyme comprises a catalytic domain of a fucosyltansferase and a localization signal from $\alpha(1,3)$ galactosyltransferase whereby said nucleic is expressed in a cell wherein said chimeric enzyme is located in a cell compartment or organelle where it is able to compete for substrate with the $\alpha(1,3)$ galactosyltransferase and wherein the $\alpha(1,3)$ galactosyltransferase is located in the same compartment or organelle as said chimeric enzyme, resulting in reduced levels of a product from the $\alpha(1,3)$ galactosyltransferase wherein said product is an epitope reactive with an antibody that causes hyperacute rejection.

29. (NEW) A nucleic acid encoding a chimeric enzyme, wherein said chimeric enzyme comprises a catalytic domain of a first glycosyltransferase and a localization signal of a different glycosyltransferase, said localization signal being specific for a trans Golgi and comprising a cytoplasmic tail of said different glycosyltransferase, whereby said nucleic acid is expressed in a cell wherein said chimeric enzyme is located in the trans Golgi where it is able to compete for substrate with the different glycosyltransferase and wherein the different glycosyltransferase is located in the same compartment or organelle as said chimeric enzyme, resulting in reduced levels of a product from said different glycosyltransferase, wherein said product is an epitope reactive with an antibody that causes hyperacute rejection.

REMARKS

No new matter is added by the amendments to the claims. Claims 1-15, 17-24 and 26-28 are currently pending in this application.

Disapproval of the Proposed Drawing Changes

The proposed drawing corrections submitted on July 14, 2000 were disapproved. Applicants respectfully submit herewith "Proposed Changes to the Drawings" along with marked-up copies of the Figures.

Antecedent Basis -- Specification

The specification was objected to as failing to provide proper antecedent basis for the claimed subject matter. Specifically, the Examiner objects to the phrases "which cause it to be recognized as non-self by the recipient" and "wherein the carbohydrate is recognized as non-self by a species" in claims 19 and 25. Claim 25 has been canceled. Therefore, the rejection is now moot with respect to this claim. Applicants respectfully submit that the amendments to claim 19 overcome this objection.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 1-14 and 16-25 were rejected under 35 U.S.C. § 112, second paragraph, as indefinite. Applicants respectfully submit that the amendments to the claims overcome this objection.

Claims 1 and 17-19 (and dependent claims) were rejected for reciting the phrase "a second glycosyltransferase." The claims have been amended to recite "a different glycosyltransferase." Applicants respectfully request withdrawal of this rejection.

Claims 1, 18 and 19 (and dependent claims) were rejected for reciting "located in an area of the cell where it is able to compete for substrate." The claims have been amended to recite that the chimeric enzyme is located "in a cell compartment or organelle where it is able to compete for substrate with the different glycosyltransferase" and that the different glycosyltransferase "is located in the same compartment or organelle as said chimeric enzyme." Applicants respectfully request withdrawal of this rejection.

Claims 3, 5, 8 and 10 were rejected for reciting the phrase "wherein the localization signal is based on, or is similar to that of...". The claims have been amended to recite the term "from" instead. Applicants respectfully request withdrawal of this rejection.

Claim 6 was rejected for reciting the phrase "galactosyl sulphating enzyme. This phrase has been deleted for the claims. Applicants respectfully request withdrawal of this rejection.

Claim 7 was rejected for reciting the phrase "originates from." Applicants have amended claim 7 to recite "are from." Applicants respectfully request withdrawal of this rejection.

Claim 8 was rejected for reciting "is intended to transform." Claim 8 has been amended to recite that the cell "belongs to the same species as the cell of claim 1." Applicants respectfully request withdrawal of this rejection.

Claims 9 and 16 (and dependent claims) were rejected for reciting "gal transferase". The claims have been amended to recite "α(1,3)-galactosyltransferase." Applicants respectfully request withdrawal of this rejection.

Claim 23 (and dependent claims) were rejected for reciting the phrase "nucleic acid that expresses a nucleic acid according to claim 1." Claim 23 has been amended to recite an expression unit "that expresses a nucleic acid according to claim 1." Applicants respectfully request withdrawal of this rejection.

Claim 25 was rejected as being an omnibus claim. Claim 25 has been canceled. Therefore this rejection is now moot.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 1-10 and 12-25 were rejected under 35 U.S.C. § 112, first paragraph, as not enabled. The Examiner indicates that the specification is only enabling for specific chimeric glycosyltransferases, such as gtHT and pgtHT, and not the scope of chimeric glycosyltransferases of claims 1, 6, 7, 8 and 17-19. Applicants respectfully traverse this rejection.

Although applicants maintain that the claims were enabled as written, to further prosecution, the claims have been amended to recite that the product is an epitope reactive with antibodies that cause hyperacute rejection.

Furthermore, Applicants submit herewith a faxed copy of a Declaration of Mauro Sergio Sandrin, which sets forth details of experiments showing that chimeric glycosyltransferases other than those specifically exemplified in the application are able to reduce the gal epitope in accordance with the invention. The signed original will be submitted to the United States Patent Office shortly. Applicants respectfully request withdrawal of this rejection.

Rejections under 35 U.S.C. § 102(a)

Claims 1-4, 12-15, 17-20 and 23 remain rejected under 35 U.S.C. § 102(a) as being anticipated by *Schwientek et al.* Applicants respectfully traverse.

Before addressing the rejection, Applicants will first provide a brief discussion of N-glycan biosynthesis. Briefly, N-glycan biosynthesis begins with production of oligosaccharides in the Rough Endoplasmic Reticulum (RER). The oligosaccharides are transferred from the RER to the Golgi complex for further processing. During synthesis, glycans are converted to hybrid and complex N-glycans by the production of "antennae." The most common antenna is formed by Gal-Tf or EC2.4.1.38. Elongation of the antennae is terminated by addition of Gal, Fuc and sialyl residues. See section 2.1, pages 90-91 and 96 in "Molecular Glycobiology" (1994) IRL Press at Oxford University Press, New York, M Fukuda and O Hindsgaul (eds), submitted herewith.

The claimed invention is directed towards the use of a chimeric enzyme having a catalytic domain of a first glycosyltransferase and a cytoplasmic domain of a localization signal from a "different" glycosyltransferase. According to the invention, the localization signal directs the chimeric enzyme to a cell compartment where it is able to compete for substrate with the "different" glycosyltransferase. Surprisingly, the inventors have found that the cytoplasmic region has the ability to target the first glycosyltransferase chimera to a Golgi site where it is able to compete with the "different" glycosyltransferase for a substrate.

The glycosyltransferases of the claimed invention are involved in the termination of antennae elongation (see discussion of N-glycan biosynthesis above). Thus, the glycosyltransferase used in the invention results in a product that is "an epitope reactive with an antibody that causes hyperacute rejection."

Nothing in Schwientek et al. teach or suggest the claimed invention. Schwientek et al. describe fusion of the membrane anchor region of α(1,2)-mannosyltransferase (Mnt1p) with human Gal-Tf (EC2.4.1.38). Gal-Tf is a glycosyltransferase involved in the synthesis of N-glycans. Whereas the Gal-Tf glycosyltransferase taught by Schwientek et al. are involved in antennae formation, the glycosyltransferases of the claimed invention are involved in the termination of antennae elongation. Thus, the Schwientek et al. glycosyltransferase does not provide "an epitope reactive with an antibody that causes hyperacute rejection."

Additionally, Schwientek et al. does not teach the use of a cytoplasmic domain of Mnt1p to direct Gal-Tf to the Golgi. Rather, Schwientek et al. disclose a fusion protein of "the membrane anchor region" (See Schwientek et al., page 3400, first paragraph on the right hand column, lines 5-6 and page 3404, last paragraph on the left hand column, first sentence). As

discussed in Applicant's previous response, a membrane anchor region is distinct from a cytoplasmic region. The Examiner is referred to page 11 of Applicant's previous Response, where the generic structure of Type II proteins is represented, and which clearly shows that the cytoplasmic tail and transfer membrane regions are separate and distinct.

Furthermore, Schwientek et al. do not teach the use of the Mnt1p-Gal-tf fusion molecule to "provide a means for outcompeting the native glycosyltransferase", as alleged by the Examiner. The α(1,6) and α(1,3) "determinants" referred to by the Examiner were not the result of competition between two enzymes, but were used to show that the Mnt1 membrane anchor region in the fusion protein gave rise to a product or structure typical of an early Golgi compartment. This merely served to indicate that the fusion protein had not reached distal Golgi sites. See page 3401, last sentence of the first, part-paragraph of the left- hand column, and the abstract. In contrast, the glycosyltransferases of the present invention operate at a site near Trans Golgi Network (TGN). As shown in page 57 of the reference given in 1 above (copy attached), the TGN is a distal Golgi network compartment. Thus, the Gal-Tf as taught by Schwientek would not compete with the glycosyltransferases contemplated by the present invention.

Finally, Applicants are unaware of any publications describing another glycosyltransferase against which the Gal-Tf taught in Schweintek would compete for substrate.

Applicants respectfully request withdrawal of this rejection.

Rejections under 35 U.S.C. § 102(b)

Claims 15 and 16 were rejected under 35 U.S.C. § 102(b) as anticipated by *Sandrin et al.* (Xenotransplatation, 1:81-88, 1994) and *Sandrin et al.* (WO 94/21799). Applicants respectfully traverse this rejection.

Claim 15 has been amended to depend from new claim 1. Claim 1 recites a chimeric enzyme of the invention and includes a localisation signal comprising a cytoplasmic region of a first enzyme. The citations do not teach such a feature. Therefore, Applicants respectfully submit that claim 15 is not anticipated by the cited references and request withdrawal of this rejection.

CONCLUSION

In view of the amendments and remarks made herein, it is respectfully submitted that the application is in condition for allowance. Notification to that effect is earnestly requested.

Respectfully submitted,

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Dated: March 3, 2003

By: _____
Melissa Jean Pytel
Reg. No. 41,512

VERSION WITH MARKINGS TO SHOW CHANGES MADE IN THE CLAIMS

Please amend claims 1-3, 5-15, 17-19, 23 and 24 and cancel claims 16 and 25 and add new claims 26-29 as follows:

1. (AMENDED) A nucleic acid encoding a chimeric enzyme, wherein said chimeric enzyme comprises a catalytic domain of a first glycosyltransferase and a localization signal of a different [second] glycosyltransferase, said localization signal comprising a cytoplasmic tail of said different glycosyltransferase, whereby said nucleic acid is expressed in a cell wherein said chimeric enzyme is located in a cell compartment or organelle [in an area of the cell] where it is able to compete for substrate with the different [second] glycosyltransferase and wherein the different glycosyltransferase is located in the same compartment or organelle as said chimeric enzyme, resulting in reduced levels of a product from said different [second] glycosyltransferase, wherein said product is an epitope reactive with an antibody that causes hyperacute rejection.

2. (AMENDED) The nucleic acid according to claim 1, wherein said localization signal localizes said catalytic domain thereby to enable the catalytic domain to compete with said different [second] glycosyltransferase for a substrate.

3. (AMENDED) The nucleic acid according to claim 1, wherein the localization signal is from [based on, or is similar to that of] a glycosyltransferase which produces glycosylation patterns which are recognised as foreign by a transplant recipient.

4. (AMENDED) The nucleic acid according to claim 1, wherein the localization signal comprises the amino terminus of the different [second] glycosyltransferase.

5. (AMENDED) The nucleic acid according to claim 1, wherein the localization signal is from α(1,3)-glycosyltransferase.

6. (AMENDED) The nucleic acid according to claim 1, wherein the first glycosyltransferase is selected from the group consisting of H-transferase, secretor, and sialytransferase [, a galactosyl sulphating enzyme and a phosphorylating enzyme].

7. (AMENDED) The nucleic acid according to claim 1, wherein the catalytic domain and the localization signal are each [originates] from a mammal selected from the group consisting of human, primates, ungulates, dogs, mice, rats and rabbits.

8. (AMENDED) The nucleic acid according to claim 1, wherein the localization signal is from a cell that belongs to [based on, or is similar to that of] the same species as the cell of claim 1 [which the nucleic acid is intended to transform].

9. (AMENDED) The nucleic acid according to claim 1, comprising a sequence encoding the catalytic domain of H transferase and a nucleic acid sequence encoding a localization signal from α(1,3)-galactosyltransferase [Gal-transferase], which transferase catalyses the production of an epitope reactive with an antibody to thereby cause hyperacute rejection.

10. (AMENDED) The nucleic acid according to claim 9, wherein the catalytic domain and the localization signal are from [based on, or are similar to that of] pigs.

11. (AMENDED) The [A] nucleic acid according to any one of claims 1 to 10, which encodes the NH2 terminal cytoplasmic tail of GT attached to the transmembrane, stem and catalytic domains of Ht [gtHT as defined herein].

15. (AMENDED) A nucleic acid according to claim 1, wherein the [An isolated nucleic acid molecule encoding a] localization signal [of a glycosyltransferase] is selected from the group consisting of MNVKGR, MNVKGK and MVVKGK.

16. (CANCELLED).

17. (AMENDED) A method of producing the nucleic acid according to claim 1, comprising the step of operably linking a nucleic acid sequence encoding a catalytic domain from a first glycosyltransferase to a nucleic acid sequence encoding a localization signal of a different [second] glycosyltransferase, said localization signal comprising a cytoplasmic tail of said different glycosyltransferase.

18. (AMENDED) A method of reducing [the level] an amount of a carbohydrate exhibited on [the] a surface of a cell, said method comprising causing a nucleic acid to be expressed in said cell [wherein said nucleic acid to be expressed in said cell] wherein said nucleic acid encodes a chimeric enzyme which comprises a catalytic domain of a first glycosyltransferase and a localization signal of a different [second] glycosyltransferase, said localization signal comprising a cytoplasmic tail of said different glycosyltransferase, whereby said chimeric enzyme is located in a cell compartment or organelle [in an area of the cell] where it is able to directly compete for substrate with said different [second] glycosyltransferase, and wherein said different [second] glycosyltransferase is located in the same compartment or organelle as said chimeric enzyme, resulting in reduced levels of a product from said different glycostyltransferase [capable of transferring said carbohydrate] ,wherein said product is an epitope reactive with an antibody that causes hyperacute rejection.

19. (AMENDED) A method of producing a cell from a donor species which is immunologically acceptable to a recipient species by reducing levels of carbohydrate on said cell, wherein said carbohydrate is capable of stimulating recognition of the cell [which cause it to be recognized] as non-self by the recipient, said method comprising causing a nucleic acid to be expressed in said cell wherein said nucleic acid encodes a chimeric enzyme which comprises a catalytic domain of a first glycosyltransferase and a localization signal of a different [second] glycosyltransferase, said localization signal comprising a cytoplasmic tail of said different glycosyltransferase, whereby said chimeric enzyme is located in a cell compartment or organelle [in an area of the cell] where it is able to directly compete for substrate with said different [second] glycosyltransferase, and wherein said different [second] glycosyltransferase is located in the same compartment or organelle as said chimeric enzyme, resulting in reduced levels of a

product from said different glycostyltransferase [capable of transferring said carbohydrate] ,wherein said product is an epitope reactive with an antibody that causes hyperacute rejection.

23. (AMENDED) An expression unit [which expresses a nucleic acid] that expresses a nucleic according to claim 1, which when used to transform a cell results in a cell which is immunologically acceptable to an animal having reduced levels of a carbohydrate on its surface, which carbohydrate is recognized as non-self by said species.

24. (AMENDED) [The expression unit according to claim 23, selected from the group consisting of a] A retroviral-packaging cassette, retroviral construct or retroviral producer cell comprising the expression unit according to claim 23.

25. (CANCELLED).

26. (NEW) A nucleic acid encoding a chimeric enzyme, wherein said chimeric enzyme comprises

a catalytic domain of a first glycosyltransferase or a carbohydrate modifying enzyme, and

a localization signal of a different glycosyltransferase, said localization signal comprising a cytoplasmic tail of said different glycosyltransferase,

whereby said nucleic is expressed in a cell wherein said chimeric enzyme is located in a cell compartment or organelle where it is able to compete for substrate with the different glycosyltransferase and wherein the different glycosyltransferase is located in the same compartment or organelle as said chimeric enzyme, resulting in reduced levels of a product from said different glycosyltransferase, wherein said product is an epitope reactive with an antibody that causes hyperacute rejection.

27. (NEW) A nucleic acid according to claim 26, wherein the carbohydrate modifying enzyme is selected from the group consisting of a galactosyl sulphating enzyme and a phosphorylating enzyme.

28. (NEW) A nucleic acid encoding a chimeric enzyme, wherein said chimeric enzyme comprises a catalytic domain of a fucosyltansferase and a localization signal from α(1,3) galactosyltransferase whereby said nucleic is expressed in a cell wherein said chimeric enzyme is located in a cell compartment or organelle where it is able to compete for substrate with the α(1,3) galactosyltransferase and wherein the α(1,3) galactosyltransferase is located in the same compartment or organelle as said chimeric enzyme, resulting in reduced levels of a product from the α(1,3) galactosyltransferase wherein said product is an epitope reactive with an antibody that causes hyperacute rejection.

29. (NEW) A nucleic acid encoding a chimeric enzyme, wherein said chimeric enzyme comprises a catalytic domain of a first glycosyltransferase and a localization signal of a different glycosyltransferase, said localization signal being specific for a trans Golgi and comprising a cytoplasmic tail of said different glycosyltransferase, whereby said nucleic acid is expressed in a cell wherein said chimeric enzyme is located in the trans Golgi where it is able to compete for substrate with the different glycosyltransferase and wherein the different glycosyltransferase is located in the same compartment or organelle as said chimeric enzyme, resulting in reduced levels of a product from said different glycosyltransferase, wherein said product is an epitope reactive with an antibody that causes hyperacute rejection.